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Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens

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ABSTRACT

Seagrasses have a long history of being used for a variety of remedial purposes, such as the fever, skin diseases, muscle pains, wounds and stomach problems. Hence it is essential to study their bioactive metabolites and medicinal properties when considering their food applications. The chemical composition of six seagrasses were determined and evaluated for their potential to urinary tract infection bacteria (UTI). The chemical composition determined by GC–MS yielded 24 compounds. For the first time 4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl- (6.68%), D-allose (3.67%) and 5-Caranol, *trans*, *trans*-(+)- (2.14%) were identified from *Halodule pinifolia*. D-Allose is a aldo-hexose (sugar) used as a potential inhibitor of glycosidases and low-calorie carbohydrate sweeteners. Among the six seagrasses tested, *H. pinifolia* and *Cymodocea rotundata* exhibited predominant growth inhibitory activity against all the UTI bacteria. This study shows the presence of various biological metabolites in tested seagrasses that can be used effectively in food and pharmacological industries.

1. Introduction

Seagrasses are submerged marine angiosperms growing abundantly in tidal and subtidal areas of all seas except in the Polar Regions. Seagrass biomass is used as human food especially by coastal populations Hemminga & Duarte, 2000. In folk medicine, seagrasses have been used for a variety of remedial purposes, e.g., for the treatment of fever and skin diseases, muscle pains, wounds, stomach problems, remedy against stings of different kinds of rays and tranquillizers for babies (de la Torre-Castro & Rönnbäck, 2004). In India, seagrasses were used as medicine (treatment of heart conditions, seasickness), food (nutritious seeds), fertilizer (nutrient rich biomass) and livestock feed (goats and sheep) (Newmaster et al., 2011). Seeds of *Enhalus acoroides* are thought to have aphrodisiac and contraceptive properties (Aliño et al., 1990). Seagrasses produce antimicrobial compounds that may act to reduce or control the microbial growth and there are many reports describing antibacterial (Ragupathi Raja Kannan, Arumugam, & Anantharaman, 2010a; Sreenath Kumar, Sarada, Gideon, & Rengasamy, 2008), antiviral (Rowley et al., 2002), anti-inflammatory (Hua et al. 2006), antidiabetic (Gokce & Haznedaroglu, 2008) and antioxidant (Ragupathi Raja Kannan, Arumugam, & Anantharaman, 2010b; Ragupathi Raja Kannan, Arumugam, Meenakshi, & Anantharaman, 2010c; Ragupathi Raja Kannan, Arumugam, Grignon Dubois, & Anantharaman, 2012; Athiperumalsamy, Devi Rajeswari, Hastha Poorna, Kumar, & Louis Jesudass, 2010) activities of bioactive compounds isolated from seagrasses.

However recent reports on the phytochemical constituents of seagrasses of the Gulf of Mannar, South India are limited except for a few reports (Athiperumalsami, Venkatraman Kumar, and Louice Jesudass, 2008; Ragupathi Raja Kannan, Arumugam, Hemalatha, & Anantharaman, 2010d). Evidences on marine antimicrobial compounds for urinary tract infection are scanty and no detailed reports on the GC–MS profiling of Indian seagrasses have been reported. In this context, the present work reports the GC–MS profiling of bioactive metabolites from aqueous methanolic extracts of the six seagrasses of the Gulf of Mannar (India) and their potential antibacterial effects on clinically important UTI bacteria.

2. Materials and methods

2.1. Seagrasses

The fresh leaves of *Enhalus acoroides* (Linnaeus f.) Royle, *Thalassia hemprichii* (Ehrenberg) Ascherson, *Halodule pinifolia* (Miki) den Hartog, *Syringodium isoetifolium* (Ascherson) Dandy, *Cymodocea serrulata* (R. Brown) Ascherson & Magnus and *Cymodocea rotundata* Ehrenberg & Hemprich ex Ascherson were collected from Chinnapallam, Gulf of Mannar Biosphere Reserve, Tamilnadu, India (Latitude 8°35′–9°25′N; 78°08′–79°30′E) during March 2010 and

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identified following the keys of Ramamurthy, Balakrishnan, Ravikumar, & Ganesan (1992). They were immediately brought to the laboratory in plastic bags containing seawater to prevent evaporation. The seagrasses were washed thoroughly with tap water to remove all sand particles and epiphytes, shade dried at room temperature (35 ± 2 °C) for a week until a constant weight was obtained and ground well using a mixer grinder. The grounded seagrasses were then stored individually in airtight containers for further use.

2.2. Preparation of seagrass extracts

Crude extracts were obtained by soaking 100 g DW of each seagrass powder individually in 2 L of aqueous methanol (1:4) for 24 h at room temperature under dark conditions. The extraction was repeated thrice, pooled and filtered through Whatmann No. 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure using a rotary flash evaporator. The dry aqueous extracts were lyophilized and stored in a refrigerator until further analysis.

2.3. Antibacterial susceptibility test

UTI bacterial pathogens (*Escherichia coli*, *Proteus mirabilis*, *Staphylococcus saprophyticus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Serratia* sp.) used in this study were obtained from Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamilnadu, India. Crude extracts of seagrasses were tested individually against all the UTI bacterial strains. The UTI bacterial strains were individually inoculated in nutrient broth and incubated for 24 h before being used for the antimicrobial assay. All the UTI bacterial strains were seeded individually in Muller Hinton Agar plates. The standard disc diffusion method described by Bauer, Kirby, Sherris, and Tvrok (1966) was followed for the antibacterial assay. The sterile disc (Hi media, India) were impregnated with 50 μg/disc of the individual crude extract, dried, placed on the already prepared agar plates and incubated for 24 h at 37 °C. Sterile discs devoid of extracts were also maintained as the control. The whole assay was carried out under sterile conditions in triplicate. The zone of inhibition was measured (mm) from the edges of the discs to the clear zone.

2.4. Minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the 96 wells microtitre plate (bacteriostatic concentration). The assay was started with the inoculation of same density of bacteria (2×10^8 cells/ml) using the table of Amsterdam (1996). Each seagrass extract (100 μl) with the concentrations of 0.01, 0.1, 1.0, 10, 25, 50 and 100 μg/ml were poured individually in 6 wells of 96 well plate for each bacterial assay. In addition, 6 wells free of extracts were used as a control. These plates were dried under U.V in a chamber for 2 h to evaporate the solvent under sterile conditions. Bacterial solutions (100 μl) were then added under aseptic conditions and the plates were incubated for 48 h at 37 °C to allow bacterial growth. One plate was used for each test bacteria to decrease the risk of contamination. The lowest concentration of extract where no turbidity was observed in 4–6 wells over 6 wells was determined and noted as the minimum inhibitory concentration (MIC) (Marechal et al., 2004).

2.5. Minimum bacterial concentration (MBC)

MBC is defined as the lowest concentration where no bacterial growth is observed (bacteriocidal concentration). This was determined from the broth dilution resulting from the 96 well MIC plates by sub culturing test bacteria in nutrient agar plates. In this technique, the contents of the microtitre plates resulting from MIC assay were streaked using a sterile wire loop on a sterile nutrient agar plate and incubated at 37 °C for 48 h. The lowest concentration of the extract which showed no bacterial growth was noted and documented as the MBC (Vollekova, Kostalova, & Sochorova, 2001; Usman, Abdulrahman, & Ladan, 2007).

2.6. GC/MS analysis

The aqueous methanol extracts of the six seagrasses were examined for their chemical composition by GC–MS engine model (GC Clarus 500, PerkinElmer and Computer Mass Spectral Library (NIST 2005) for more than 80,000 compounds. The capillary column Elite-1 (100% Dimethyl poly siloxane) 30 × 0.25 mm × 1 μm df. The extracts were diluted in methanol and the injection volume for samples was 1 ml injected in the split mode with 10:1. Electron ionisation (EI) mass spectra were measured at 70 eV over the mass range 45–450. The chromatographic conditions were helium used as a carrier gas and 250 °C injector temperature. The column oven temperature was maintained at 110 °C for 2 min, then increased to 200 °C at the rate of 10 °C/min, MS total time was 44 min. The constituents were identified after comparison with data available in the computer library attached to the GC–MS instruments and reported in the literature.

3. Results and discussion

3.1. Antibacterial activity

Among the six seagrasses, *H. pinifolia* and *C. rotundata* exhibited inhibitory activity against all the UTI bacteria. *H. pinifolia* showed the bioactivity as zone of inhibition ranging from 10.3 ± 1.53 to 14.3 ± 1.15 mm and the antagonistic activity of *C. rotundata* was between 9.7 ± 0.58 and 12 ± 1.0 mm. Moderate levels of antagonistic activity were registered by *T. hemprichii* and *E. acoroides*. *T. hemprichii* showed the bioactivity against all the test strains and was in the range from 9.3 ± 0.57 to 11.3 ± 1.15 mm whereas *E. acoroides* showed growth inhibitory activity from 6.3 ± 0.58 to 9.3 ± 1.53 mm but did not inhibited the growth of *E. coli* and *S. saprophyticus*. *S. isoetifolium* and *C. serrulata* were found to be resistant against most of the UTI pathogens and their antagonistic activity was the lowest (Table 1). MIC and MBC were estimated for all the seagrass extracts over the UTI bacteria. *H. pinifolia* inhibited the growth of test bacteria with the MIC of 1.0 μg/ml and its MBC was 25 μg/ml (full data was not shown). On the other hand, the lowest bioactivity was exerted by *C. serrulata* and its MIC was 100 μg/ml and no bactericidal activity was noticed within the concentrations tested (Table 2).

Urinary tract infection remains the most common reason for outpatients to seek medical care and for inpatients to develop nosocomial infections. Antimicrobial properties of seagrasses have been reported by several authors. Alam et al. (1994) found the methanolic extract of *E. acoroides* were more effective against *S. aureus, K. pneumoniae* and *P. aeruginosa* than the hexane extract. Sreenath Kumar et al. (2008) reported *Halophila* and *Zostera* were more effective than *Cymodocea*. The lipid and water-soluble phenolic extracts of leaf and root-rhizome fractions of *H. pinifolia* showed a strong antibacterial activity (Balasubramanian, Kannan, & Thangaradjou, 2000). These results are in agreement with the present finding where *H. pinifolia* showed maximum antagonistic activity. Extract of *H. pinifolia* inhibited the growth of bacteria with least concentration (MIC) of 1.0 μg/ml and killed (MBC) the test bacteria completely at 25 μg/ml. Recently, Ragupathi Raja Kannan et al. (2010a) also reported similar findings where the methanol

Table 1
Antibacterial activity of seagrasses extracts against UTI pathogens. Results are presented as mean ± SE (n = 3).

Name of the UTI pathogens	Seagrasses extracts					
	E. acoroides	*T. hemprichii*	*H. pinifolia*	*S. isoetifolium*	*C. serrulata*	*C. rotundata*
E. coli	R	9.3 ± 0.57	12.3 ± 1.53	R	R	10 ± 1.0
P. mirabilis	9.3 ± 1.53	10.3 ± 1.15	13.7 ± 0.58	8.7 ± 1.15	R	12 ± 1.0
S. saprophyticus	R	9.3 ± 0.57	10.7 ± 1.15	8.3 ± 0.58	6.0 ± 1.0	11.6 ± 1.52
K. pneumonia	8.3 ± 0.58	11.3 ± 1.15	11.7 ± 1.53	R	R	11.3 ± 0.57
P. aeruginosa	9.3 ± 0.57	10.6 ± 2.08	10.3 ± 1.53	R	6.3 ± 0.57	12.3 ± 0.57
E. aerogens	8.7 ± 0.58	9.3 ± 1.52	14.3 ± 1.15	7.0 ± 1.0	R	9.7 ± 0.58
Serratia sp.	6.3 ± 0.57	8.7 ± 1.15	11.3 ± 2.08	R	R	10 ± 1.0

R, resistant (no zone of inhibition).

Table 2
Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of seagrasses extracts against UTI pathogens.

Seagrasses extracts	MIC (μg/ml)	MBC[a] (μg/ml)
E. acoroides	25	100
T. hemprichii	25	50
H. pinifolia	1.0	25
S. isoetifolium	50	100
C. serrulata	100	NB
C. rotundata	10	50

NB, no bactericidal activity within the test concentrations.
[a] Observations of six replicates.

extract of *H. pinifolia* exhibited strong antibacterial activity against human pathogens.

3.2. GC/MS analysis

The qualitative and quantitative results obtained using GC–MS are presented in Table 3. In total, 24 components were identified from the six seagrasses. *H. pinifolia* recorded the highest (15) number of compounds, while *E. acoroides, T. hemprechii and S. isoetifolium* recorded seven phytoconstituents each. On the other hand, *C. serrulata* and *C. rotundata* registered the presence of ten and nine phytocomponents respectively with species level variation in relative amounts. Some of the GC–MS peaks remained unidentified because of lack of authentic standards and library data of the corresponding compounds.

Among the identified phytoconstituents, fatty acids such as tridecanoic acid, tetradecanoic acid and n-hexadecanoic acid are reported to have the antioxidant and antimicrobial activities (Bodoprost & Rosemeyer, 2007). 9,12,octadecadienoic acid (Z,Z) has anti-inflammatory and antiarthritic properties (Kalaivani, Sathish, Janakiraman, & Johnson, 2012). The 3,7,11,15-tetramethyl-2-hexadecan-1-ol is a acyclic diterpene alcohol has the property of antimicrobial and anti-inflammatory activities (Kalaivani et al., 2012). This is the first report of such compound from seagrasses. It is a fragrance producing component used as ingredient in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents (McGinty, Letizia, & Api, 2010). 3,7,11,15-tetramethyl-2-hexadecan-1-ol is being used worldwide with less than 0.01 metric tons per annum (IFRA (International Fragrance Association), 2004). For the first time, 4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl-, D-allose and 5-caranol, trans,trans-(+)- were identified from *H. pinifolia*. 4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl- is a flavonoid fraction that

Table 3
Chemical components (%) identified in the different Indian Seagrasses.

RT	Name of the compound	EA	TH	HP	SI	CS	CR
15.46	Hexadecanoic acid, methyl ester	4.96	1.54	3.96	–	3.03	2.82
16.13	n-Hexadecanoic acid	24.59	32.86	14.75	42.88	35.74	55.55
17.97	9,12-Octadecadienoic acid (Z,Z)-	2.6	0	5.76	0	12.28	17.67
18.05	9-Octadecanoic acid (Z)-. Methyl ester	15.46	37.21	0	24.04	24.32	8.56
18.38	Phytol	16.6	13.49	–	–	16.94	7.23
18.81	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	27.62	–	12.74	–	–	–
19.12	Octadecanoic acid	8.18	–	–	–	–	–
14.52	3,7,11,15-tetramethyl-2-hexadecan-1-ol	–	1.45	0.75	1.15	1.48	2.08
19.56	Oleic acid	–	2.5	–	5.51	2.25	4.09
5.74	(Z)6, (Z)9-Pentadecadien-1-ol	–	1.94	–	–	–	–
10.69	4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl-	–	–	6.68	–	–	–
13.33	D-Allose	–	–	3.67	–	–	–
5.74	5-Caranol, trans,trans-(+)-	–	–	2.14	–	–	–
15.17	7-Hexadecanoic acid, methyl ester, (Z)-	–	–	0.93	–	–	–
15.85	Hexadecanoic acid, Z-11-	–	–	2.04	–	–	–
17.95	9,12-Octadecadienoic acid methyl ester, (E,E)-	–	–	2.78	–	–	–
18.05	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	–	–	12.74	–	–	–
18.80	9,12,15-Octadecatrienoic acid (Z,Z,Z)-	–	–	22.83	–	–	–
15.46	Tridecanoic acid, methyl ester	–	–	–	1.61	–	–
18.39	Cyclopentaneundecanoic acid, methyl ester	–	–	–	3.29	–	–
18.81	13-Octadecenal, (Z)-	–	–	–	21.52	–	–
9.74	Nonane, 1-chloro-	–	–	–	–	0.85	1.55
12.20	Dedecane, 1-chloro-	–	–	–	–	0.54	0.44
13.64	Tetradecanoic acid	–	–	0.57	–	2.56	–
Total identified (%)		100	90.99	92.34	100	99.99	99.99

RT, retention time; EA, *Enhalus acoroides*; TH, *Thalassia hemprichii*; HP, *Halodule pinifolia*; SI, *Syringodium isoetifolium*; CS, *Cymodocea serrulata*; CR, *Cymodicea rotundata*.

has antimicrobial and anti-inflammatory properties. D-Allose is a rare aldo-hexose (sugar). The rare sugars which inhibit the glycosidase are now attracting attention of researchers (Muniruzzaman et al., 1996). These sugars are now widely used for preparing low-calorie carbohydrate sweeteners and as bulking agents (Livesey & Brown, 1996).

4. Conclusion

In the present study, the antibacterial activity extended by *H. pinifolia* and *C. rotundata* is very much appreciable for the future development of novel medicinal products against UTI infections. Our results clearly demonstrated the presence of 3,7,11,15-tetramethyl-2-hexadecan-1-ol, 4H-Pyran-4-one-2,3-dihydro-3,5-dihydroxy-6-methyl-, D-allose and 5-caranol, trans,trans-(+)- from the tested seagrasses. Further bioassays, purification and structural characterisation of these biological metabolites will yield noteworthy information about their usage in pharmaceuticals, cosmetics and food industry.

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References

Alam, K., Agua, T., Maven, H., Taie, R., Rao, K. S., Burrows, I., et al. (1994). Preliminary screening of seaweeds, seagrass and lemongrass oil from Papua New Guinea for antimicrobial and antifungal activity. *Pharmaceutical Biology, 32*(45), 396–399.

Aliño, P. M., Cajipe, G. J. B., Ganzon-Fortes, E., Licuanan, W. R. Y., Montaño, N. E., & Tupas, L. M. (1990). The use of marine organisms in folk medicine and horticulture: a preliminary study. *SICEN Leaflet, 1*, 1–8.

Amsterdam, D. (1996). Susceptibility testing of antimicrobials in liquid media. In: V. Loman, (Ed.) *Antibiotics in laboratory medicine*, 4th ed. Williams and Wilkins, Baltimore, MD. pp. 52–111.

Athiperumalsami, T., Venkatraman Kumar, V., & Louice Jesudass, L. (2008). Survey and phytochemical analysis of seagrasses in the Gulf of Mannar, southeast coast of India. *Botanica Marina, 51*, 269–277.

Athiperumalsamy, T., Devi Rajeswari, V., Hastha Poorna, S., Kumar, V., & Louis Jesudass, L. (2010). Antioxidant activity of seagrasses and seaweeds. *Botanica Marina, 53*, 251–257.

Balasubramanian, R., Kannan, L., & Thangaradjou, T. (2000). Screening of seagrasses for antibacterial activity against bacterial pathogens. *Seaweed Research & Utilization, 22*(1&2), 101–106.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Tvrok, M. (1966). Antibiotic susceptibility testing by standardized single disc method. *American Journal of Clinical Pathology, 45*, 493–496.

Bodoprost, J., & Rosemeyer, H. (2007). Analysis of phenacylester derivatives of fatty acids from human skin surface sebum by reversed-phase HPLC: Chromatograpic mobility as a function of physico-chemical properties. *International Journal of Molecular Science, 8*, 1111–1124.

de la Torre-Castro, M., & Rönnbäck, P. (2004). Links between humans and seagrasses-an example from tropical East Africa. *Ocean & Coastal Management, 47*, 361–387.

Gokce, G., & Haznedaroglu, M. Z. (2008). Evaluation of antidiabetic, antioxidant and vasoprotective effects of *Posidonia oceanica* extract. *Journal of Ethnopharmacology, 115*, 122–130.

Hemminga, M. A., & Duarte, C. M. (2000). *Seagrass ecology*. New York. pp: Cambridge University Press. 298.

Hua, K. F., Hsu, H. Y., Su, Y. C., Lin, I. F., Yang, S. S., Chen, Y. M., et al. (2006). Study on the anti-inflammatory activity of methanol extract from Seagrass *Zostera japonica*. *Journal of Agriculture and Food Chemistry, 54*(2), 306–311.

IFRA (International Fragrance Association). (2004). *Use level survey*, August 2004, Report No. 37011, Firmenich Inc., Princeton, New Jersey.

Kalaivani, C. S., Sathish, S. S., Janakiraman, N., & Johnson, M. (2012). GC–MS studies on *Andrographis paniculata* (Burm f) Wall. Ex Nees – A medicinally important plant.. *International Journal of Medicinal and Aromatic Plants, 2*(1), 69–74.

Livesey, G., & Brown, J. C. (1996). D-Tagatose is a bulk sweetener with zero energy determined in rats. *Journal of Nutrition, 126*, 1601–1609.

Marechal, J. P., Culioli, G., Hellio, C., Thomas-Guyon, H., Callow, M. E., Clare, A. S., et al. (2004). Seasonal variation in antifouling activity of crude extracts of the brown alga *Bifurcaria bifurcate* (Cystoseiraceae) against cyprids of *Balanus amphitrite* and the marine bacteria *Cobetia marina* and *Pseudoalteromonas haloplanktis*. *Journal of Experimental Marine Biology and Ecology, 313*, 47–62.

McGinty, D., Letizia, C. S., & Api, A. M. (2010). Fragrance material review on phytol. *Food and Chemical Toxicology, 48*, S59–S63.

Muniruzzaman, S., Pan, Y. T., Zeng, Y., Atkins, B., Izumori, K., & Elbein, A. D. (1996). Inhibition of glycoprotein processing by l-fructose and l-xylulose. *Glycobiology, 6*, 795–803.

Newmaster, A. F., Berg, K. J., Ragupathy, S., Palanisamy, M., Sambandan, K., & Newmaster, S. G. (2011). Local knowledge and conservation of seagrasses in the Tamil Nadu State of India. *Journal of Ethnobiology and Ethnomedicine, 7*, 37.

Ragupathi Raja Kannan, R., Arumugam, R., & Anantharaman, P. (2010a). Antibacterial potential of three Seagrasses against Human pathogens. *Asian Pacific Journal of Tropical Medicine, 11*, 890–893.

Ragupathi Raja Kannan, R., Arumugam, R., & Anantharaman, P. (2010b). In-vitro antioxidant activities of *Enhalus acoroides*. *Asian Pacific Journal of Tropical Medicine, 11*, 898–901.

Ragupathi Raja Kannan, R., Arumugam, R., Meenakshi, S., & Anantharaman, P. (2010c). Thin layer chromatography analysis of Antioxidant constituents of seagrasses of Gulf of Mannar. *International Journal of ChemTech Research, 2*(3), 1526–1530.

Ragupathi Raja Kannan, R., Arumugam, R., Hemalatha, A., & Anantharaman, P. (2010d). Phytochemical analysis of seagrasses from Mandapam coast, Tamilnadu. *Seaweed Research & Utilization, 32*(1&2), 171–175.

Ragupathi Raja Kannan, R., Arumugam, R., Grignon Dubois, M., & Anantharaman, P. (2012). Antioxidant activity of seagrasses of the Mandapam coast, India. *Pharmaceutical Biology, 50*(2), 182–187.

Rowley, D. C., Hansen, M. S. T., Rhodes, D., Sotriffer, C. A., Ni, H., McCammon, J. A., et al. (2002). Thalassiolins A–C: New Marine – Derived Inhibitors of HIV cDNA integrase. *Bioorgonic Medicinal Chemistry, 10*, 3619–3625.

Ramamurthy, K., Balakrishnan, N. P., Ravikumar, K., & Ganesan, R. (1992). Seagrasses of Coromandel Coast, India, Flora of India Series 4, *Botanical survey of India*. p. 80.

Sreenath Kumar, C., Sarada, D. V. L., Gideon, T. P., & Rengasamy, R. (2008). Antibacterial activity of three South Indian seagrasses, *Cymodocea serrulata*, *Halophila ovalis* and *Zostera capensis*. *World Journal of Microbiology and Biotechnology, 24*(9), 1989–1992.

Usman, H., Abdulrahman, F. I., & Ladan, A. H. (2007). Phytochemical and antimicrobial evaluation of *Tribulus terrestris* L (Zygophylaceae). Growing in Nigeria. *Research Journal of Bioscience Medwell Journals, 2*(3), 244–247.

Vollekova, D., Kostalova, D., & Sochorova, R. (2001). Isoquinoline Alkaloids from Mahoniaaquifolium stem bark is active against Malassezia Sp. *Folia Microbiology, 46*, 107–111.